

CLAIMS

1. A method of selecting a switching system, the switching system comprising: (i) a first component comprising a first molecule and (ii) a second component comprising a second molecule, in which the first molecule binds to the second molecule in a manner modulatable by a ligand, and (iii) a third component comprising the ligand, the method comprising the steps of:
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- (a) contacting one or more candidate first molecules with one or more candidate second molecules in the presence of one or more ligands;
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- (b) selecting a complex of the three components;
- (c) optionally isolating and/or identifying the unknown components of the complex;
- (d) comparing the binding of the first molecule component of the complex to the second molecule component of the complex in the presence and absence of the ligand component of the complex; and
- 15
- (e) selecting complexes where said binding differs in the presence and absence of the ligand component,
- in which at least one component is provided in the form of a library of members.
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2. A method according to Claim 1, in which at least one of the candidate first molecules comprises a non-naturally occurring binding domain which binds to the second molecule.
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3. A method according to Claim 1 or 2, in which one of the first molecule and second molecule comprises a nucleic acid binding molecule, and the other of the first and second molecules comprises a nucleic acid.
4. A method according to Claim 1, 2 or 3, in which one or both of the candidate nucleic acid and nucleic acid binding molecules is provided as a plurality of molecules.
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109

TCAGCTGACTTCGGATGCATTAGATGACTTTGACTTAGATATGCTAGGATCTGACGCGCTA
GACGATTTGATCTGGACATGTTGGGCAGCGATGCTCTGGACGATTTGATTTAGATATGC
TTGGCTCGGATGCCCTGGATGACTTCGACCTCGACATGCTGTCAAGTCAGCTGAGCCAGGA
ACAAAAACTTATTTCTGAAGAAGATCTGTAAGGATCC

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Sequence ID 3: TFIIIA/Zif binding site

TgcgtgggcgTGTACCTggatgggagacC

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05996494-42804
TGCCTTCTGAAGAAGATCTGTAAGGATCC

5. A method according to Claim 4, in which the nucleic acid binding molecule is provided as a library of nucleic acid binding molecules.
6. A method according to Claim 4 or 5, in which a single target nucleic acid is used.
7. A method according to Claim 4, 5 or 6, in which one of the components isolated and/or identified in step (c) is a ligand component.
8. A method according to any of Claims 4 to 7, in which one of the components isolated in step (c) is a nucleic acid binding molecule component.
9. A method according to any of Claims 4 to 8, in which the nucleic acid is provided as a library of nucleic acid sequences. the sequences being related to one another by sequence homology.
10. A method according to any of Claims 4 to 9, in which a plurality of candidate ligands are used.
11. A method according to any of Claims 4 to 10, in which the ligands are provided as a library of ligands.
12. A method according to any of Claims 4 to 11. in which the candidate nucleic acid binding molecules are polypeptides.
13. A method according to any of Claims 4 to 12, in which the polypeptides are at least partly derived from DNA binding proteins, preferably transcription factors.
14. A method according to any of Claims 4 to 12, in which the candidate nucleic acid binding molecules are derived from zinc finger transcription factors.
15. A method according to any of Claims 4 to 13, in which the candidate nucleic acid binding molecules are provided as a phage display library.

16. A method according to any of Claims 4 to 14, in which the ligand is selected from Distamycin A, Actinomycin D and echinomycin.
- 5 17. A switching system comprising a gene switch, in which the switching system has been selected by a method according to any of Claims 4 to 15.
18. Use of a nucleic acid binding molecule selected by a method according to any one of Claims 4 to 15 in a method of regulating transcription from a nucleic acid sequence
10 comprising a target nucleic acid to which the nucleic acid binding molecule binds in a manner modulatable by a ligand.
19. Use of a ligand selected by a method according to any one of Claims 4 to 15 in a method of regulating transcription from a nucleic acid sequence comprising a target
15 nucleic acid to which a nucleic acid binding molecule binds in a manner modulatable by the ligand.
20. Use of a target nucleic acid selected by the method of any one of Claims 4 to 15 in a method of regulating transcription from a nucleic acid sequence comprising the target
20 nucleic acid to which a nucleic acid binding molecule binds in a manner modulatable by a ligand.
21. A method of modulating the expression of one or more genes, said method comprising administering a nucleic acid binding molecule and a ligand selected
25 according to the method of any one of Claims 4 to 15 to a cell, in which the regulatory sequences of the genes comprise a target nucleic acid selected according to a method according to any of Claims 4 to 15.
22. A method of modulating the expression of one or more nucleotide sequences of
30 interest in a host cell which host cell comprises a nucleic acid sequence capable of directing the expression of a nucleic acid binding molecule and a target nucleic acid sequence to which the nucleic acid binding molecule binds in a manner modulatable

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by a ligand, which method comprises administering said ligand to the cell and wherein the nucleic acid binding molecule is heterologous to the host cell.

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23. A method according to Claim 21 or 22 wherein the host cell is a plant cell.
24. A method according to Claim 23, in which the plant cell is part of a plant and the target sequence is part of a regulatory sequence to which the nucleotide sequence of interest is operably linked, said regulatory sequence being preferentially active in the male or female organs of the plant.
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25. A non human transgenic organism comprising a target nucleic acid sequence and a nucleic acid sequence capable of directing the expression of a nucleic acid binding molecule which binds to the target nucleic acid in a manner modulatable by a ligand, in which the target nucleic acid sequence and/or nucleic acid sequence are
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- heterologous to the organism.
26. A transgenic non-human organism according to Claim 25 which is a plant.
27. A method according to Claim 1 or 2, in which each of the first and second molecules
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- comprises a polypeptide.
28. A method according to Claim 27, in which the first molecule comprises a polypeptide binding protein and the second molecule comprises a polypeptide.
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29. A method according to Claim 27 or 28, in which one or both of the first and second molecules is provided as a library of polypeptides.
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30. A method according to Claim 27, 28 or 29, in which the ligands are provided as a library of ligands.
31. A method according to any of Claims 27 to 30, in which the ligand is an immunoglobulin molecule, preferably an antibody molecule.

32. A method according to any of Claims 27 to 31, in which the first molecule is a nucleic acid binding protein capable of binding to nucleic acid.
- 5 33. A method according to Claim 32, in which the nucleic acid binding protein binds to nucleic acid in a manner modulatable by the second molecule.
34. A switching system comprising a protein switch, in which the switching system has been selected by a method according to any of Claims 27 to 33.
- 10 35. Use of a nucleic acid binding protein selected by a method according to Claim 33, in a method of regulating transcription from a nucleic acid sequence comprising a target nucleic acid to which the nucleic acid binding protein binds.
- 15 36. Use of a ligand selected by a method according to Claim 33, in a method of regulating transcription from a nucleic acid sequence comprising a target nucleic acid to which the nucleic acid binding protein binds in a manner modulatable by the ligand.
- 20 37. Use of a target nucleic acid selected by the method according to Claim 33, in a method of regulating transcription from a nucleic acid sequence comprising a target nucleic acid to which the nucleic acid binding protein binds in a manner modulatable by a ligand.
- 25 38. A method of modulating the expression of one or more genes, said method comprising administering a nucleic acid binding protein and a ligand selected according to a method according to Claim 33 to a cell, in which the regulatory sequences of the genes comprise a target nucleic acid to which the nucleic acid binding protein binds in a manner modulatable by a ligand.
- 30 39. A method of modulating the expression of one or more nucleotide sequences of interest in a host cell which host cell comprises a first nucleic acid sequence capable

of directing the expression of a nucleic acid binding protein, a second nucleic acid sequence capable of directing the expression of a second polypeptide, the binding between the nucleic acid binding to the second polypeptide being modulatable by a ligand, and a target nucleic acid sequence to which the nucleic acid binding protein binds in a manner modulatable by a second polypeptide, which method comprises administering said ligand to the cell.

40. A method according to Claim 39, in which the nucleic acid binding protein is heterologous to the host cell.

41. A method according to Claim 39 or 40 wherein the host cell is a plant cell.

42. A method according to Claim 42, in which the plant cell is part of a plant and the target sequence is part of a regulatory sequence to which the nucleotide sequence of interest is operably linked, said regulatory sequence being preferentially active in the male or female organs of the plant.

43. A non human transgenic organism comprising a target nucleic acid sequence, a first nucleic acid sequence capable of directing the expression of a nucleic acid binding protein, and a second nucleic acid sequence capable of directing the expression of a second polypeptide which binds to the nucleic acid binding protein in a manner modulatable by a ligand, in which the nucleic acid binding protein binds to the target nucleic acid sequence in a manner modulatable by binding of the second polypeptide.

44. A method according to Claim 43, in which any or all of the first nucleic acid sequence, the second nucleic acid sequence, and the target nucleic acid sequence are heterologous to the organism.

45. A transgenic non-human organism according to Claim 43 or 44 which is a plant.

46. A method according to any of Claims 1 to 16 and 27 to 33, in which the first molecule component of the complex has a higher affinity for the second molecule

component of the complex in the presence of the ligand component than in the absence of the ligand component.

- 5 47. A method according to of Claims 1 to 16 and 27 to 33, in which the first molecule component of the complex has a higher affinity for the second molecule component of the complex in the absence of the ligand component than in the presence of the ligand component.

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